

EXHIBIT B
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appl. No.	:	10/067,185	Confirmation No. 5257
Applicant	:	Thomas D. Johnson	
Filed	:	February 1, 2002	
TG/A.U.	:	1651	
Examiner	:	Leon B. Lankford	
Docket No.	:	02-0201-JOHN	
Customer No.	:	26357	

DECLARATION UNDER RULE 132

I, THOMAS D. JOHNSON, do hereby declare and say:

My residence address is Buffalo, South Dakota.

I am the inventor of the invention claimed in the subject application.

I am the owner and president of TJ Technologies, Inc. I founded TJ Technologies in 1978. Our headquarters is in Buffalo, SD, which is located in the northwest corner of South Dakota. TJ Technologies, Inc. also owns and operates a state of the art research and development laboratory

located in Brookings, SD. Our mission statement is: "To gain a more complete understanding of the creation in which we live, through systematic scientific research, so we can positively influence the profitability and sustainability of production agriculture through innovative and consistently effective agriculture products."

I have been in the business of soils research for the purpose of product development since 1982. I was the Principal Investigator of the research project that discovered the claimed invention in the performance of National Science Foundation Small Business Innovative Research (SBIR) Award No. DMI-9901629. Microbiology research was established as a research objective of TJ Technologies, Inc. in 1985 and has remained a primary objective to the present. I have published works in the Proceedings of the 19th, 20th and 24th National Sunflower Research Forums (1997, 1998, & 2002).

Based on the above description of my background, I am an ordinary practitioner of the art of biocontrol.

I have studied the references cited by the Examiner and I am of the opinion that the claims are not obvious in view of those references. The examiner has suggested that example 2 of Shanmuganathan patent (5,525,132) teaches combining bacteria and fungi, but example 2 of patent number 5525132 does not teach the combining of organisms. In fact the, Table 1 referred to in the example, shows only individual organisms. This example teaches away from my invention in that there is no suggestion that combining non-performing organisms will improve their performance, nor does it suggest that combining the D9 organism with any other will improve its performance or consistency. Neither does it suggest that any treatment caused

increased nutrient intake or yield response, only pathogen control. Moreover, the D9 organism is a yeast and is to be applied to the foliage of the plant, not the seed.

The Examiner further suggests that Neyra (5,697,186) teaches the use of multiple organisms.

This patent does not teach the combining of organisms for the purpose of increasing the effectiveness of pathogen control. In fact, it teaches a delivery method for *Rhizobium* (nitrogen fixing bacteria for legumes) as a means to increase nitrogen nodulation. It also teaches application of *Azospirillum brasilense* (free living nitrogen fixing bacteria) on turf grasses using the disclosed flocculation technology to increase the time of viability on the seed. The disclosed combinations of organisms are only combinations of bacteria (see Claim 7).

The Examiner further suggests that Ocamb (6,133,196) teaches combining fungi and bacteria for pathogen control. In this invention (as best I can determine) the bacterial component is applied to the seed, the seed is allowed to germinate and produce root and the fungal component is then added to the soil media. In addition, the organisms are both different than my invention and the plant involved is different, i.e., conifers. This patent does not teach the application of a bacteria and a fungi on the seed and then planting the seed. It teaches a basically different invention.

Further, in my opinion, this reference teaches away from my invention in that it teaches a difference in timing of application (i.e., a bacterium is applied to a seed and a fungus is applied to the soil in which the seed is planted).

Patent No. 5,413,783 (McLaughlin) teaches the use of yeasts for control of plant pathogens. The disclosed combinations are only combinations of yeasts, not combinations of fungi and bacteria.

This invention also teaches the application of the yeast to the foliage or the fruit, not the seed.

Yeasts are in the kingdom-Fungi, division-Ascomycota, which is the same for *Trichoderma*. The classes differ however. *Trichoderma* is in the class Deuteromycota (Fungi Imperfecti) and yeasts are in the class Hemiascoycetes. Yeasts reproduce by fission or budding. *Trichoderma* reproduce by the formation of conidia and yeasts do not do. *T. virens* is multicellular and yeasts are unicellular. Given the uncertainties involved in the biocontrol art, experience with biocontrol combinations comprising yeasts and do not teach how to make combinations comprising *T. virens* to a person having ordinary skill in the art.

Nonobviousness determinations must recognize that biocontrol is an unpredictable art. In an article cited in the Background Art section of the subject application, there is this statement: “However, these results demonstrate the difficulty in obtaining significantly reliable results among these microorganisms for biological control under field conditions. This lack of reproducibility is not unexpected as commonly an organism used for biological control is taken from one environment and expected to act in another to which it is adapted” (Whipps, 1992). This quote is from a document published by the University of Florida and entitled *Effect of Bacterial and Fungal Microorganisms to Colonize Tomato Roots, Improve Transplant Growth and Control Fusarium Crown and Root Rot*, by L. E. Datnoff and K. L. Pernezny, University of Florida, Everglades Research and Education Center, Belle Glade, Florida. This trial looked at *Gliocladium (Trichoderma) virens* GL 21 and *Trichoderma harzianum* alone and in combination with undisclosed bacterial strains. Because of the documented unpredictability of the biocontrol art, it is impossible to predict how combinations of microorganisms will perform without conducting extensive experimentations as we have done. This University of Florida study

documented the biocontrol inconsistency problem and confirmed the problem with a citation from Whipps.

In an enclosed article by the Ohio State Extension Service entitled “Some soil bacteria protect soybeans from diseases” the author argues the importance of finding bacteria that are indigenous to Ohio. In essence, Ohio State Extension Service is pointing out that the effects of biocontrol organisms should not be effective across geography. The surprising results achieved by the claimed invention shows that this conclusion is incorrect for the applicant’s discovery.

In a paper entitled Mechanism in the biocontrol of *Rhizoctonia solani* – induced cotton seedling disease by *Gliocladium virens*: antibiosis by C. R. Howell and R. D. Stipanovic, Phytopathology 85 469-472, 1995, the authors discuss the fact that *Gliocladium virens* (*Trichoderma virens* and *Gliocladium virens* are synonymous) produces a substance called “gliotoxin.” In this paper, the organism that was used to test for the presence or absence of gliotoxin production was *Bacillus subtilis* because gliotoxin inhibits the growth of *Bacillus subtilis*. The first full paragraph on p. 472 of the reference states:

“HPLC analysis of extracts from mutants of *G. virens* that failed to produce clear zones in lawns of *B. subtilis* showed that the mutants were consistently deficient for gliotoxin production. Since gliotoxin inhibits *B. subtilis* (1), and none of the other secondary metabolites known to be produced by *G. virens* have been demonstrated to have activity against it or other *Bacillus* spp., this system appears to be rather specific for detecting gliotoxin production or a deficiency for it in this fungus.”

Because the *Trichoderma virens* Gl-3 of the claimed invention is known to produce gliotoxin and it has remained in its wild state in preferred claimed embodiments, it is not only nonobvious that a strain of *Bacillus subtilis* var. *amyloliquifaciens* would produce a synergistic effect, one skilled in the art would understand that this reference teaches away from the claimed combination as an effective means to produce a synergistic effect. Thus, the applicant's invention not only produces surprising results by creating a synergistic effect between the two organisms, the claimed invention also solves several problems were encountered in the prior art.

One surprising aspect of the invention is that it solves long-felt but unsolved needs and overcomes the failure of others. Specifically, the claimed invention does not contain a bacterium that has been implicated in complicating cystic fibrosis and the claimed invention does contain a spore-forming bacterium, allowing seeds coated with the claimed combination to be stored much longer before planting.

W. Mao, R. D. Lumsden, J. A. Lewis and P. K. Hebbar published a paper in 1998 entitled Seed treatment using pre-infiltration and biocontrol agents to reduce damping-off of corn caused by species of *Pythium* and *Fusarium*, Plant Disease, 82:294-299. In this trial, they combined *Trichoderma virens* isolate Gl-3 and *Burkholderia cepacia* strains Bc-B and Bc-1. The trials were conducted with each organism alone and combined fungal/bacterial combinations. The results of the trial showed the combination better than either organism alone, but a serious problem remained.

I determined that a product containing *Burkholderia cepacia* would not be able to be commercialized because it was implicated as complicating lung conditions in cystic fibrosis patients. Some of the complications included death. Obviously that organism would not be viable in a commercial product.

I screened numerous bacteria, both gram negative and gram positive, and discovered that the *Bacillus* TJ 1000 was not only antagonistic to the target pathogens, but that it was compatible with the *T. virens* Gl-3. The results of field trials showed that it was not only compatible, but it was also synergistic with *T. virens* Gl-3.

It should be noted that *Bacillus subtilis* var. *amyloliquefaciens* strain TJ 1000 is antagonistic to most fungi. It is NOT antagonistic to the fungi *Trichoderma virens* Gl-3. That is significant, rare, and also nonobvious in my opinion. Furthermore, in a paper published in Applied and Environmental Microbiology, Dec. 1979, p. 1120-1126, William D. Rosensweig and G. Stotzky confirm that "In general, the antagonistic activity of bacteria towards filamentous fungi was greater in soil than on agar." Thus, all of the prior art points away from this combination producing an improved response over either one alone.

The discovery that a particular gram positive bacterium (a spore-forming bacterium) is compatible with *T. virens* is highly significant. The gram negative bacterium *Burkholderia cepacia* was problematic in the application to a seed. Because it does not produce a spore form, it had to be applied to the seed with Pelgel and had to be planted within 5 days. The Pelgel would hold sufficient moisture (17%) to keep the *Burkholderia* from dying due to dehydration.

However, the Pelgel also would hold enough moisture that it would swell the seed and germination was often reduced significantly.

The utilization of the Bacillus TJ 1000 allows application of the claimed combination of organism to the seed without retaining any increased moisture on the seed coat. As a result, the biologically treated seed can be stored for at least 90 days if not more without damage to the microorganisms or the seed. This is only possible because of the discovery of this otherwise nonobvious combination.

This is evidence that there were several problems with the use of a gram negative bacterium. However, use of a gram positive bacterium was not considered feasible by others because the prior art taught that the *T. virens* would antagonize gram positive bacteria. Thus, the claimed invention is nonobvious because it solves long-felt but unsolved needs and overcomes the failure of others.

Another surprising aspect of my claimed invention is that plants that grow from seeds treated with my claimed combination experience increased uptake of manganese. The protective nature of increased manganese uptake is documented in Project S-269: Biological Control and Management of Soilborne Plant Pathogens for Sustainable Crop Production, 5th International Conference on the Biogeochemistry of Trace Elements. July 11-15 1999. Vienna, Austria, p. 1086-1087. Dr. Don Huber of Purdue University has documented the connection between an imbalance in the ratio of nitrogen to manganese and the incidence of stalk rot in corn. (Huber D. 2000. "Hidden Hunger" threatens many crops. Purdue News. Online at:

www.purdue.edu/UNS/html4ever/0012.Huber.deficiency.html or

www.news.uns.purdue.edu/UNS/html4ever/0012.Huber.deficiency.html

The claimed combination of *Trichoderma virens* and *Bacillus amyloliquifaciens* for the purpose of plant pathogen control and increased plant yield has some very unexpected characteristics.

The first is the fact that the combination produces an increase in yield, not just plant protection from the pathogen. Plant tissue analysis from test plots presented in tables 1 and 2 below show an unexpected trend toward higher nutrient intake of a nutrient, manganese.

The treatments that produced the surprising results shown in Table 1 are defined as follows:

bs-unt-bt = Brookings, SD location – no treatment on the seed – Bt variety of corn

bs-max-bt= Brookings, SD location –chemical fungicide Maxim on the seed –Bt variety of corn

bs-1000-bt= Brookings, SD location –*Bacillus amyloliquifaciens* TJ 1000 on the seed– Bt variety

bs-0300-bt=Brookings, SD location-*Trichoderma virens* Gl-3 on the seed – Bt variety of corn

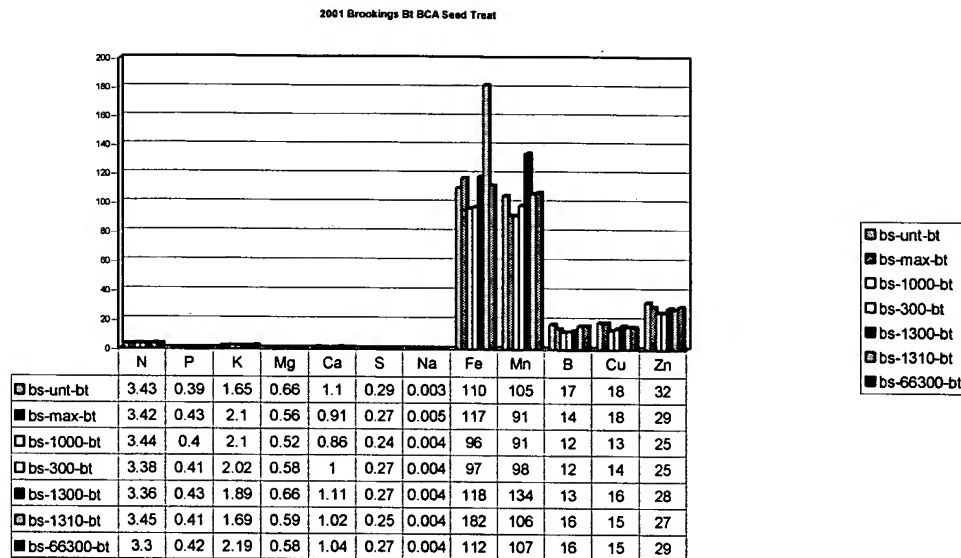
bs-1300-bt= Brookings, SD location- *B. amyloliquifaciens* TJ 1000 and *T. virens* Gl-3 (1 to 1 ratio) on the seed – Bt variety of corn (one of the claimed combinations)

bs-1310-bt = Brookings, SD location – *B. amyloliquifaciens* TJ 1000 and *T. virens* Gl-3 (7 to 3 ratio) on the seed – Bt variety of corn

bs-066300-bt = Brookings, SD location – *B. lentimorbus* and *T. virens* Gl-3 (1 to 1 ratio) on the seed – Bt variety of corn

The term “Bt” is defined as: A corn hybrid that has been genetically modified by the insertion of a gene from the bacteria *Bacillus thuringensis*. The inserted gene produces a protein that will kill European corn bore that feed on the plant tissue.

Table 1. Effects of Treatments on Plant Mineral Content on Bt Variety of Corn at Brookings SD
Location



The treatments that produced the surprising results in Table 3 are defined as follows:

bl-unt-non = Brookings location – no treatment on the seed – non Bt variety of corn (non Bt can also be described as: non genetically modified)

bl-max-non = Brookings location – chemical fungicide Maxim on the seed – non Bt variety of corn

bl-1000-non = Brookings location – *Bacillus amyloliquifaciens* TJ 1000 on the seed – non Bt variety of corn

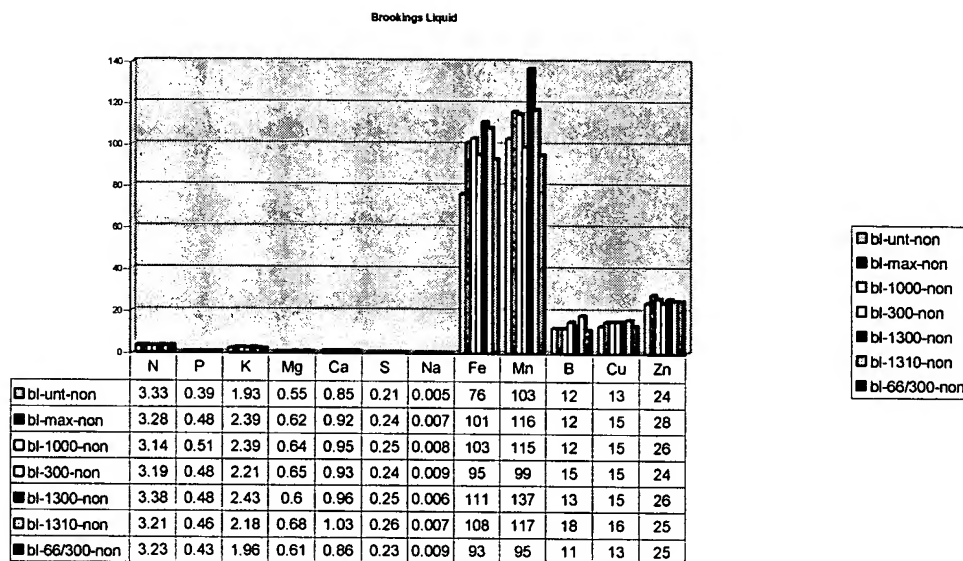
bl-300-non = Brookings location – *Trichoderma virens* Gl-3 on the seed – non Bt variety of corn

bl-1300-non = Brookings location – *B. amyloliquifaciens* TJ 1000 and *T. virens* Gl-3 on the seed (1 to 1 ratio) – non Bt variety of corn (one of the claimed combinations)

bl-1310-non = Brookings location – *B. amyloliquifaciens* TJ 1000 and *T. virens* Gl-3 on the seed (7 to 3 ratio) – non Bt variety of corn

bl-066300-non = Brookings location – *B. lentimorbus* and *T. virens* Gl-3 on the seed (1 to 1 ratio) – non Bt variety of corn

Table 2. Effects of Treatments on Plant Mineral Content on Non Bt Variety of Corn at Brookings SD Location



Manganese is known in the art as a disease prevention micronutrient. However, if manganese is added to fertilizer and applied to corn, the expected result is a decrease in yield. The significance of my claimed invention is that it increases the manganese content of the corn plant while increasing yield. Furthermore, the increase in the manganese content in the plant does not occur with either organism alone or when the *Trichoderma virens* is combined with a different organism (e.g., treatment 66/300) or the formulation of the mixture is altered (e.g., treatment 1310). This increase in manganese content of the plant tissue is documented in tables 1 and 2

above on Bt (genetically modified) corn and conventional (non-genetically modified) corn.

Tissue analysis of the corn in the charts above was done after the silking and pollination of the corn, documenting that this increase in manganese continues into the late stages of growth. Late season intake is significant because the lack of manganese in the plant is implicated in mid to late season stalk rot.

Data from claimed combinations of the Trichoderma with other bacteria strains show that other combinations tested did not increase the manganese levels to the level of the present invention. It is surprising that neither organism alone increased the manganese level in the tissue of the corn. Only seed treatment with the claimed combination of the *T. virens* Gl-3 fungus and the *B. amyloliquifaciens* bacterium increase the manganese level in the tissue of both the Bt and non-Bt corn.

Another surprising aspect of my claimed invention is unexpected consistency of increased yield: (1) consistency compared to either organism alone, in that our field trial results show the claimed combination to be significantly higher in yield over the control in both individual locations and multiple location and either organism alone did not produce a significant yield response over the control; (2) consistency across geography, in that our field trial results show the combination to be effective in a number of geographies from North Dakota to Arizona; and (3) consistency of higher yield in a more than one crop, in that our field data collected on corn, soybeans, sunflowers and wheat show significant increased in yield with the claimed combination. Our field trial results are presented in the working examples in the subject application. The results of

our field trials produced a surprisingly consistent yield response, and consistency is what is commercially important.

The claimed combination of microorganisms gives more consistent yield response than either microorganism alone. The claimed combination produces a consistent increase in yield over a range of conditions while alone the microorganisms do not. The data in the patent application show this, but the data presented in Table 3 below that was produced at the experiment station in Carrington, ND sum it up well.

Table 3. Consistency of Yield Response

	2000	2001	2002	3 YR
Control	96.9	146	87.7	110.2
Bacillus	93.3	150	94.9	112.7
T. virens	94.7	162	88.5	115.1
QuickRoot	105.6	156	90.4	117.3
1310	89.5	151	88.5	109.6

In Table 3, the treatments are defined as follows:

Control = chemical fungicide Maxim

Bacillus = *B. amyloliquifaciens* alone

T. virens = *T. virens* Gl-3 alone

Quick Root = QuickRoots™ is the product name of the claimed combination of *T. virens* Gl-3 and *B. amyloliquifaciens*

1310 = *T. virens* Gl-3 and *B. amyloliquefaciens* at a 7:3 ratio.

The column headings in Table 3 denote the year of the trial with “3YR” indicating the average treatment response for the combined three years. Note that in 2000, seed treatment with the individual organisms alone (the individual components of the claimed combination) produced yields that were less than control. In 2001, seed treatment with individual organisms both produced yields that were greater than the control as did the claimed combination. In 2002, seed treatment with the individual organisms produced yields that were greater than the control and again the claimed combination increased yield as well.

The North Dakota data presented in Table 3 document consistency in two of ways. First, in reviewing year 2000 data, neither the *Bacillus* bacteria (1000) seed treatment nor the *Trichoderma* fungi (GI-3) seed treatment by themselves produced a positive yield response; but the claimed combination did produce a positive response. Two negative responses added together do not produce a positive. Synergism is what creates positive response from two negatives. In years 2001 and 2002, the performance of treatments with the bacteria and the fungi traded places as the top seat while the performance of the claimed combination performed between treatments with the individual components. Overall, the consistent performance of the claimed combination gave the largest yield advantage because of consistency of response. These data are from the same location; only weather changed from season to season. The *Bacillus* alone seed treatment did not perform well at all in the average and the *Trichoderma* alone seed treatment only averaged well because it had one great performance out of three.

Presented in Table 4 is a compilation of data from three years of field trials, 63 entries, at 12 locations. The test plots were located at North Dakota State University, University of Arizona, and Colorado State University. This compilation clearly shows the 50/50 combination of *B. amyloliquefaciens* + *T. virens* (one of the claimed combinations) produces a significantly higher yield than the control and than either organism alone. It should be noted that while the individual components show a numerical increase in yield, it is a non-significant increase at a 0.05 rejection level while the claimed combination is significant at a 0.05 rejection level.

The Examiner has suggested that the combining of the two components is simply the additive effect of the two ingredients. While that argument may make sense in some fields of science, it does not make sense here. In the field of agronomy, a plant may respond to a specific compound at a given rate of application. Adding twice as much of that compound or adding two like compounds at the recommended rate does not add twice as much response as a rule. For example: The control in this example is the chemical fungicide Maxim. If twice as much Maxim as the recommended rate were applied to the seed, one trained in the art would expect a yield decrease from too much compound on the seed not a yield increase. In the same vain, adding recommended rates of the chemical fungicide Maxim and a similar chemical fungicide Captan on the same seed would also be expected by some skilled in the art to decrease yield, not increase it. The art of biocontrol is similar to these examples in that, once an application rate of microbiological component is established, it is understood by those skilled in the art that adding more numbers of microorganism or adding more species of microorganism for a similar purpose will not automatically increase yield and may, in fact, reduce it.

Table 4. QuickRoots™ Effect on Corn Yield in Replicated Field Trials.

3 Year Average Evaluating QuickRoots™/Maxim vs. Maxim

Treatment	Moisture	Yield	Pricing	Advantage
Control	17.5	154.77	\$300.25	
<i>B. amyloliquefaciens</i> alone	17.5	158.7	\$307.88	\$7.62
<i>T. virens</i> alone	17.4	158.81	\$308.57	\$8.31
<i>B. amyloliquefaciens</i> + <i>T. virens</i> combined 50/50	17.5	161.62	\$313.54	\$13.29
Mean	17.5	158.88	\$307.56	
CV (%)	23.3	21.7		
LSD (0.05)	.19(NS)	5.05		

Based on the evidence presented in this declaration and in the application as filed, it is my professional opinion that the combinations I have claimed produce unexpected results. These unexpected results render my claimed combinations patentable.

Another line of evidence shows that products that embody the invention have achieved commercial success because of the presence in the products of the claimed combination. Specifically, TJ Technologies, Inc., a business owned by the applicant has received a \$397,890 Phase II and a \$350,000 Phase IIB Small Business Innovation Research (SBIR) award from the National Science Foundation for field trials of the claimed combination after a feasibility study performed under a \$99,473 Phase I SBIR award proved the feasibility of the concept. Both of these awards were made in a national competition for Federal research and development funding.

The commercial success of the claimed combination is also shown by the success of a test market conducted in November and December of 2003. The test market was conducted to test the market acceptance of this microbiology combination on corn. The test market was conducted in cooperation with REA Hybrids of Aberdeen, South Dakota. During the test market phase, the benefits of the microbiology combination seed treatment was explained and then offered as an option the buyer could choose as a treatment on the seed purchase. The product retail cost is \$15.00 per bag of seed. Approximately 6,500 bags of seed (representing approx. 20,000 acres) were sold by REA Hybrids during this test phase. Of the 6,500 bags of seed sold 4,635 bags were ordered with the microbiology seed treatment. That represents 72 percent of the seed sold. This test market illustrates the tremendous need and acceptance for the solution this product brings to the agriculture industry. No factor other than the benefits of the claimed invention is responsible for this commercial success, and, thus, a nexus exists between the claimed invention and the commercial success.

TJ Technologies, Inc. incorporates the claimed combination in a product named *QuickRoots™* that is currently registered for sale in IA, MT, NE, ND, SD, WY. QuickRoots™ will be limited to an additional 20,000 acres of available product for 2004 spring sales due to production limitations. Production capabilities are being increased and sales volumes are expected to increase dramatically in 2005.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application and any patent issued thereon.

Signed: *Thomas D. Johnson*

Thomas D. Johnson

Dated: *3/15/04*